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Original article

Labile soil organic matter fractions as influenced by non-flooded mulching cultivation and cropping season in rice–wheat rotation

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ABSTRACT

Labile soil organic matter (SOM) fractions are especially important because they are more vulnerable to disturbance and play a crucial role for nutrient and carbon cycling. Although water conservation has become increasingly important in rice–wheat rotation, the effects of non-flooded mulching cultivation on labile SOM fractions remain unknown. Based on a long-term field experiment (10 years), we analyzed the impact of non-flooded mulching cultivation and cropping season on labile SOM fractions in a rice–wheat rotation in Chengdu Plain, southwest China. Compared with traditional flooding (TF), the plastic film mulching (PM) and wheat straw mulching (SM) treatments increased dissolved organic carbon (DOC) (42% after rice season and 41% after wheat season), but decreased microbial biomass carbon (MBC) (19% after rice season and 28% after wheat season) and nitrogen (MBN) (17% after rice season and 24% after wheat season) in the 0–5 cm depth. SM increased particulate organic carbon (POC) and $KMnO_4$-oxidizable C ($KMnO_4$-C) contents after both the rice and wheat seasons. Microbial biomass and DOC concentrations were higher for all three cultivations after the rice season than after the wheat season. In contrast, POC contents under PM and SM were higher after the wheat season than after the rice season. In general, results in this study indicate that non-flooded mulching and cropping season significantly influenced labile SOM fractions. The DOC fraction was the most sensitive fraction affected by non-flooded mulching, while POC and PON fractions respond fast within the two cropping seasons.

1. Introduction

Rice–wheat rotations are one of the most important production systems in China, presently occupying a total area of 13 million ha in the Yangtze River Basin [1]. Irrigated rice production consumes large quantities of irrigation water; however, freshwater for irrigation is now becoming scarce due to increasing competition from urban and industrial demand [2]. This has threatened the sustainability of irrigated rice systems and, therefore, water-saving cultivation techniques such as non-flooded mulching cultivation involving plastic film and crop residues have increasingly been introduced.

Soil organic matter (SOM) plays an important role in the long-term productivity of agroecosystems because it directly and indirectly affects soil chemical, physical and biological properties, in turn affecting crop productivity [3]. Recent attention has focused on SOM storage of agroecosystems, in particular because of concerns over atmospheric CO_2 levels and global warming [4]. This calls for maintaining satisfactory SOM levels for sustainable agroecosystems and environmental protection. Aerobic soil conditions under non-flooded mulching cultivation are favorable for SOM decomposition, so conversion from traditional flooding to non-flooded mulching may affect the SOM level. Previous studies have reported that plastic film mulching (PM) can lead to a short-term decrease in total soil organic carbon (SOC) and total nitrogen (TN) [5]. Long-term field experiments by Liu et al. [6] and Fan et al. [7,8], however, revealed that, compared with traditional flooding (TF), non-flooded mulching in rice–wheat rotation can lead to similar (PM) or increased (wheat straw mulching: SM) SOM levels. These authors attributed this to physical protection of soil C and N as a result of increased soil aggregation capacity and increased residue input in non-flooded mulching cultivation [8]. Nevertheless, short- and medium-term SOC changes in response to management practice are difficult to detect due to higher background levels and natural soil variability [9].

Labile SOM fractions such as microbial biomass C (MBC) and N (MBN), dissolved organic C (DOC), particulate organic C (POC) and N

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(PON) and $KMnO_4$-oxidizable C ($KMnO_4$-C) are characterized by their rapid turnover and are recommended as early indicators of the effects of management practices on soil quality [9]. Meanwhile, labile SOM fractions are also a major source for C and N loss. Positive relationships between initial DOC concentrations and CO_2 release were observed [10]. Several other authors have found close correlation between DOM concentration and denitrification potentials or rates [11,12]. In a field study of paddy soil, higher CH_4, CO_2 and N_2O emissions were linked to higher MBC, $KMnO_4$-C amounts following the application of rice straw + green manure as compared with control treatment [13]. Accordingly, measuring these labile SOM fractions is essential to identify changes in SOM quality and environmental sustainability.

A unique feature of low land rice–wheat systems is the annual conversion of soil from aerobic to anaerobic conditions and then back to aerobic conditions [6]. Differences in soil environment and cropping species in rice–wheat rotations could yield major effects on labile SOM fractions. E.g. Microbial biomass was increased under wet season while decreased under dry season in semiarid woodland [14]. Residue input or crop species may affect particulate organic matter (POM) or $KMnO_4$-oxidizable carbon ($KMnO_4$-C) because they reflect relatively younger organic compounds such as residues as well as small or shallow roots [15,16]. Non-flooded mulching cultivation for rice alters the soil environment (e.g. soil temperature, soil redox condition and residue input) and leads to a prolonged aerobic phase compared with the traditional flooding system. However, how these changes effect on seasonal dynamics in SOM fractions in rice–wheat rotations remain unknown.

As part of our evaluation of non-flooded mulching cultivation, this study was designed to (1) reveal the influence of converting from traditional flooding to non-flooded mulching on total SOM and especially the labile SOM fractions, and (2) evaluate the dynamics of labile SOM fractions under different cropping seasons. This information will be useful for understanding the soil organic matter dynamics and developing integrated management practices to improve soil organic matter in rice–wheat rotations.

2. Materials and methods

2.1. Study site description

The field experiment was conducted on a fluvaquent, calcareous gray floodplain soil at Wenjiang district, Sichuan province, China (30° 42′ N and 103° 50′ E). The region is classified as humid subtropical zone with a monsoon climate; the mean annual temperature and precipitation are 16.2 °C and 950 mm, respectively.

2.2. Field experiment

The field experiment, started in 1999, was described previously in detail by Liu et al. [6]. The experiment had a rice (*Oryza sativa* L.) and wheat (*Triticum* aestivum L.) rotation with three treatments: traditional flooding cultivation as control, plastic film mulching (PM) and wheat straw mulching (SM) as the two non-flooded mulching cultivation treatments. Each treatment consisted of 150 kg N ha^{-1} as $CO(NH_2)_2$, 40 kg P ha^{-1} as $Ca(H_2PO_4)_2$ and 75 kg K ha^{-1} as K_2SO_4 for rice; for wheat the values were 120 kg N as $CO(NH_2)_2$, 26 kg P ha^{-1} as $Ca(H_2PO_4)_2$ and 50 kg K ha^{-1} as K_2SO_4. There were three replicates of each treatment and the plot size of each was 3 × 8 m^2.

Every year in late May, the land was puddle and leveled. Plastic film (0.005 mm thick, 1.7 m wide) was used to cover the soil in the PM treatment. Wheat straw, harvested from the wheat season, was used to cover the soil uniformly in the SM treatment. Then rice was transplanted using two seedlings per hill at 20 × 28 cm spacing in all three systems. The rice was harvested from ground level manually by sickle in mid-September. Wheat was sown in later October and was harvested in mid-May of the following year.

For water management in the rice season, the plots of the TF treatment were irrigated every 3–5 days to maintain a 3 cm water level until 2 weeks before the rice harvest. For the PM and SM treatments, however, no permanent water layer was maintained on the soil surface during rice growth; only limited irrigation was provided from the transplanting (late May) to flowering stage (early August) when the soil moisture content fell below 80% of field capacity.

2.3. Soil sampling and analysis

Soil samples were collected from three depths (0–5 cm, 5–12 cm, 12–24 cm) within the three treatments after the rice harvest in September 2009 and the wheat harvest in May 2010. Five soil cores were taken in each plot and mixed to give 1 composite sample per field. The samples were immediately transported to the laboratory and stored at 4 °C.

A subsample was air-dried first, pretreated with 0.5 mol L^{-1} HCL to remove carbonate, then oven dried at 60 °C, ball-milled and analyzed for SOC and total nitrogen (TN) by dry combustion using an EA1108 CHN elemental analyzer (Fisons Instruments, Germany).

MBC and MBN were analyzed using a modified chloroform-fumigation–extraction method [17]. Briefly, fresh soil samples equivalent to 20 g air-dried soil were fumigated at 25 °C for 24 h. After removing the $CHCl_3$, C and N were extracted from the fumigated and non-fumigated samples with 0.5 mol L^{-1} K_2SO_4 (soil/solution ratio of 1:4 w/v) for 1 h. The filtered extracts were analyzed using a Multi 3100 N/C TOC analyzer (Analytik Jena, Germany). A K_C value of 0.45 and a K_N value of 0.54 were used to calculate the C and N content of the microbial biomass [17].

DOC was measured by the method recommended by Jones and Willett [18]. The field-moist soil samples (equivalent to 20 g oven-dried soil) were extracted with 100 ml 0.5 mol L^{-1} K_2SO_4 (soil/solution ratio of 1:5 w/v) for 1 h. The extract was then passed through a 0.45-μm membrane filter and analyzed for C using a Multi 3100 N/C TOC analyzer (Analytik Jena, Germany).

POC and PON were determined with a method modified after Cambardella and Elliott [15]. 20 g of air-dried soil <2 mm was dispersed in 100 ml of sodium hexametaphosphate ($(NaPO_3)_6$) (5 g L^{-1}) with shaking by hand during the first 15 min and then on a reciprocating shaker (180 r min^{-1}) for 18 h. The soil suspension was poured over a 53 μm sieve using a flow of distilled water. All

Table 1
Long-term effect of non-flooded mulching on soil organic carbon (SOC) and total nitrogen (TN).

Soil parameters	Soil depths (cm)	TF	PM	SM
SOC (g kg^{-1})	0–5	17.2 ± 0.16b	16.8 ± 0.06b	18.8 ± 0.94a
	5–12	16.1 ± 0.13ab	15.2 ± 0.49b	17.3 ± 0.72a
	12–24	9.34 ± 0.42a	9.79 ± 0.14a	10.2 ± 0.40a
TN (g kg^{-1})	0–5	1.97 ± 0.02ab	1.84 ± 0.03b	2.11 ± 0.07a
	5–12	1.86 ± 0.01a	1.84 ± 0.05a	1.94 ± 0.12a
	12–24	1.12 ± 0.04a	1.13 ± 0.02a	1.13 ± 0.03a
C/N	0–5	9.21 ± 0.13a	8.99 ± 0.19a	8.89 ± 0.17a
	5–12	8.68 ± 0.11a	8.04 ± 0.25a	8.96 ± 0.26a
	12–24	8.35 ± 0.11a	8.41 ± 0.21a	9.04 ± 0.30a

Standard errors of the means ($n = 3$) are presented as ± values. Means followed by different lower-case letters are significantly ($p < 0.05$) different between cultivation systems.
Abbreviations for treatments: TF = traditional flooding, PM = plastic film mulching, SM = wheat straw mulching.

material remaining on the sieve – defined as the particulate organic matter – was washed into a dry dish, oven dried at 60 °C, weighed, ball-milled and analyzed for C and N by dry combustion using an EA1108 CHN elemental analyzer (Fisons Instruments, Germany).

$KMnO_4$-C was determined following the method of Blair et al. [16] and Vieira et al. [19]: Air-dried soil samples containing 15 mg C were weighed into centrifuge tubes and allowed to react with 333 mM $KMnO_4$ for 1 h at 25 °C under tumbled shaking. After being centrifuged, the supernatants were diluted 1:250 with deionized water. The absorbance of the diluted samples and standards were read on a split beam spectrophotometer at 565 nm. The change in the $KMnO_4$ concentration is used to estimate the amount of C oxidized, assuming that 1 mM $KMnO_4$ is consumed (MnVII + MnII) in the oxidation of 0.75 mM or 9 mg of C.

2.4. Statistical analysis

The data were statistically analyzed using a one-way analysis of variance (ANOVA) with SAS (SAS Inc. 1996). Differences were considered significant at $p < 0.05$, with separation of means by the least significant difference (LSD).

3. Results

3.1. Long-term effects of non-flooded mulching on soil organic C and total N

Ten years of mulching cultivation affected SOC and TN at the 0–5 cm soil depth, but not below 5 cm (Table 1). All cultivation systems showed higher SOC and TN contents in surface soil (0–5 cm) than the 5–12 cm and 12–24 cm layers. Compared with TF, PM showed similar SOC level, whereas SM increased SOC by 9.3% at the 0–5 cm soil depth. The significant differences in TN were not found between non-flooded mulching cultivations and TF across all soil depths.

3.2. Labile SOM fractions in rice–wheat rotations under non-flooded mulching cultivation

3.2.1. Soil microbial biomass C and N

Non-flooded mulching cultivation significantly affected MBC and MBN at the 0–5 cm and 5–12 cm soil depths, while it had no effect below 12 cm (Fig. 1). Compared with TF, MBC under PM and SM significantly decreased by 17.2% and 21.4%, whereas MBN under PM and SM significantly decreased by 20.0% and 13.9% at 0–5 cm after the rice season (Fig. 1A, B). MBC and MBN followed the same trend at 5–12 cm, whereas no differences were observed in the 12–24 cm soil depth.

The soil MBC and MBN concentrations under three cultivations after the wheat season followed the same trend as after the rice season (Fig. 1C, D). Soil MBC after the rice season significantly increased by 2%, 15% and 13% than after wheat season for TF, PM and SM at 0–5 cm soil depth. Meanwhile, soil MBN concentration was also significantly higher for all three cultivation systems after rice than wheat season.

3.2.2. Dissolved organic carbon

In the topsoil (0–5 cm), DOC concentrations were 1.6 and 1.3 times higher under PM and SM when compared with TF after the

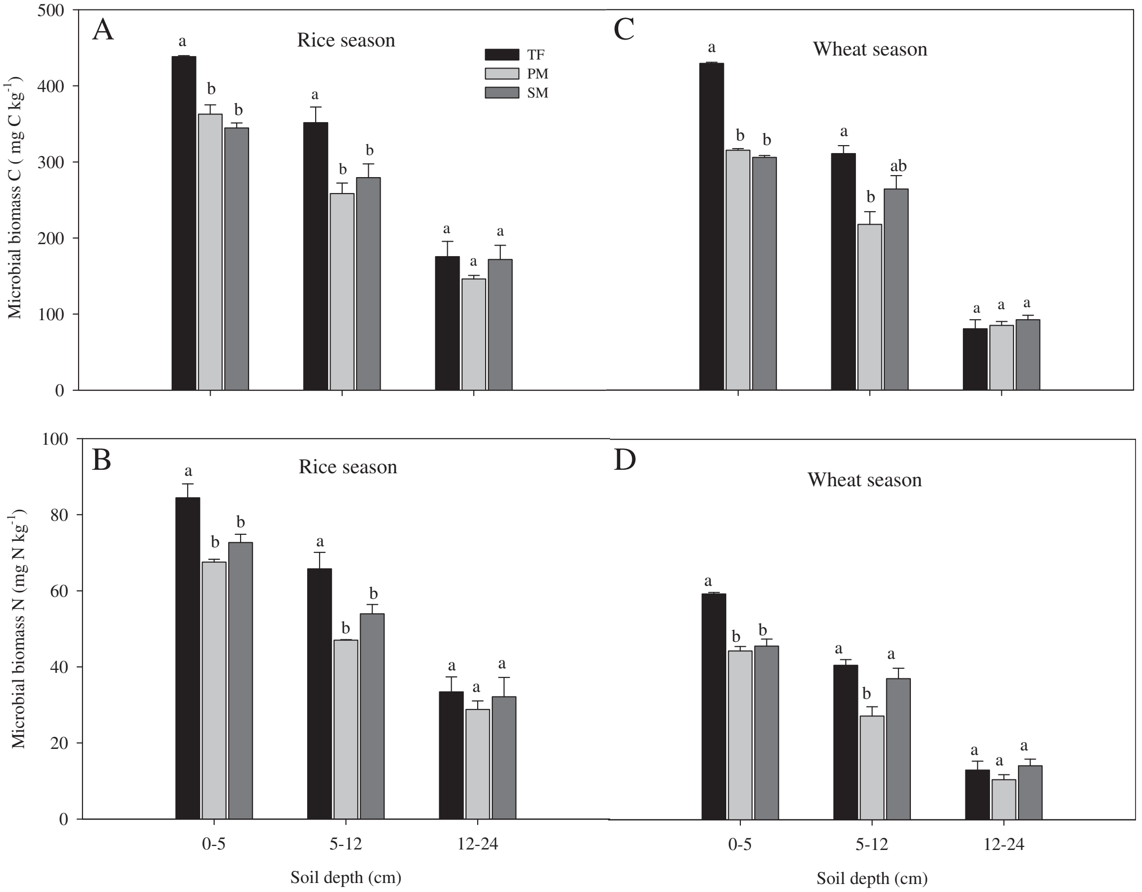

Fig. 1. Effect of mulching and soil depth on microbial biomass C and N after the rice season (A, B) and wheat season (C, D). Error bars represent standard errors of the means ($n = 3$). Means followed by different letters are significantly ($p < 0.05$) different between cultivation systems within each soil depth. Abbreviations for treatments: TF = traditional flooding, PM = plastic film mulching, SM = wheat straw mulching.

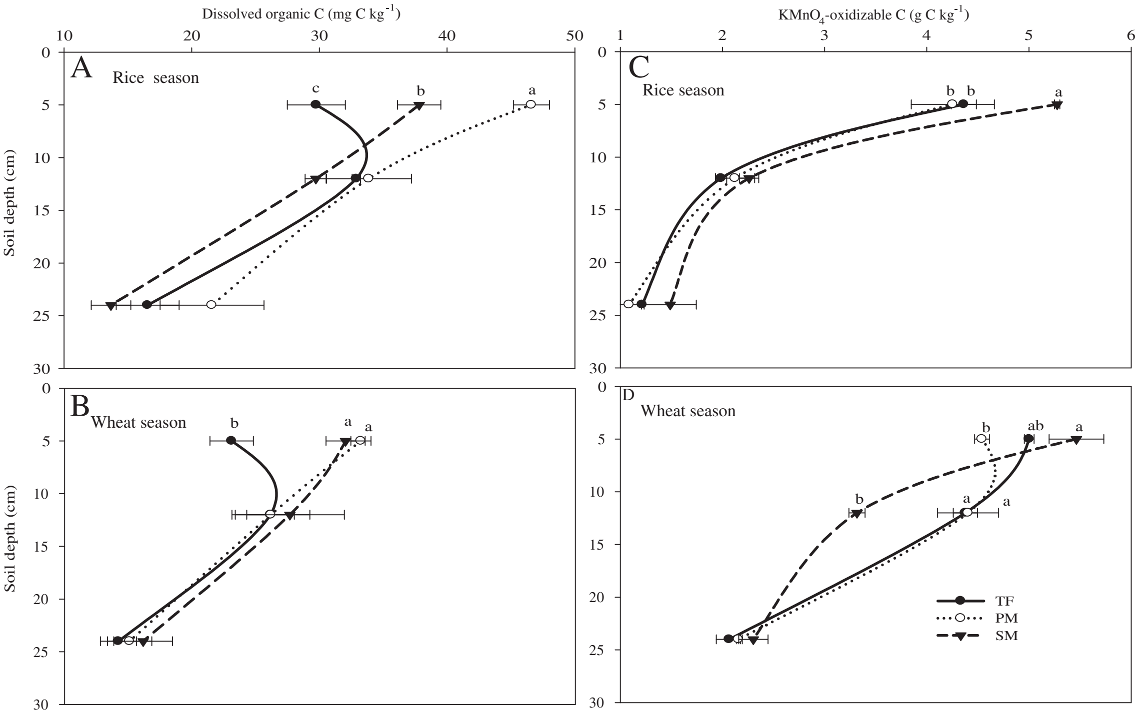

Fig. 2. Effect of mulching and soil depth on dissolved organic C and $KMnO_4$-oxidizable C after rice (A, C) and wheat seasons (B, D). Error bars represent standard errors of the means ($n = 3$). Means followed by different letters are significantly ($p < 0.05$) different between cultivation systems within each soil depth. Abbreviations for treatments: TF = traditional flooding, PM = plastic film mulching, SM = wheat straw mulching.

rice season (Fig. 2A). The DOC concentration decreased significantly with increasing soil depth under PM and SM. The DOC concentration under TF showed a considerably smaller increase at the 5–12 cm soil depth, followed by a significant decrease at 12–24 cm.

The DOC concentration under the three cultivation systems after the wheat season followed the same trend as after the rice season (Fig. 2B). Similar to MBC and MBN, the DOC concentration was also significantly higher for all three cultivation systems after the rice than after the wheat season.

3.2.3. $KMnO_4$-oxidizable carbon

Mulching cultivation had a significant effect on $KMnO_4$-C content at the 0–5 cm soil depth, but seldom below 5 cm (Fig. 2C, D). Compared with TF, SM showed significantly higher $KMnO_4$-C at 0–5 cm soil depth; PM resulted in a 2.46% lower $KMnO_4$-C after the rice season (Fig. 2C). The $KMnO_4$-C content was higher in the surface (0–5 cm) than in deeper layers (<5 cm).

The $KMnO_4$-C content under the three cultivation systems after the wheat season followed the same trend as after the rice season at the 0–5 cm soil depth (Fig. 2D). Significantly higher $KMnO_4$-C content after the wheat than the rice season was only observed under TF system in surface layer.

3.2.4. Particulate organic C and N

Compared with TF, the POC content under PM decreased by 16.4% after the rice season, whereas it increased by 6.94% under SM in the 0–5 cm soil depth (Table 2). In contrast to POC, mulching cultivation did not affect PON after the rice season. POC and PON were highest in surface soil (0–5 cm and 5–12 cm) and fell by half at 12–24 cm (data not shown).

Compared with TF, POC increased by 10.1% and 38.4% for PM and SM, respectively, after the wheat season (Table 2). Mulching cultivation also did not affect PON after the wheat season. POC contents for TF, PM and SM after the wheat season at the 0–5 cm soil depth were 9.10%, 30.9% and 29.9% higher than those after the rice season, respectively.

3.3. Relative sensitivity of C fractions in response to cultivation systems and cropping seasons

Changes in SOM fractions differed between non-flooded mulching systems (PM and SM) and TF after both the rice and wheat seasons (Fig. 3a, b). In general, relative changes of labile SOM fractions were more sensitive than total SOC and TN change. Compared with a small decline of total SOM (<8% for SOC and <4% for TN), the relative decline or increase of labile SOM fractions was

Table 2
Effect of mulching cultivation and cropping (rice and wheat) season on C content, N content, and C/N ratios in POM in 0–5 cm.

Treatment	POC (g kg^{-1})		PON (g kg^{-1})		POC/PON	
	Rice season	Wheat season	Rice season	Wheat season	Rice season	Wheat season
TF	3.60 ± 0.22a	3.96 ± 0.16b	0.27 ± 0.03a	0.32 ± 0.02a	13.6 ± 0.52b	12.4 ± 0.97a
PM	3.01 ± 0.16b	4.36 ± 0.15b*	0.22 ± 0.01a	0.36 ± 0.01a*	13.7 ± 0.08b	12.0 ± 0.02a
SM	3.85 ± 0.21a	5.48 ± 0.50a*	0.24 ± 0.01a	0.41 ± 0.06a*	15.8 ± 0.10a*	13.5 ± 0.69a

Standard errors of the means ($n = 3$) are presented as ± values. Means followed by different lower-case letters are significantly ($p < 0.05$) different between cultivation systems; means followed by * are significantly ($p < 0.05$) different from the corresponding seasons.
Abbreviations for treatments: TF = traditional flooding, PM = plastic film mulching, SM = wheat straw mulching.

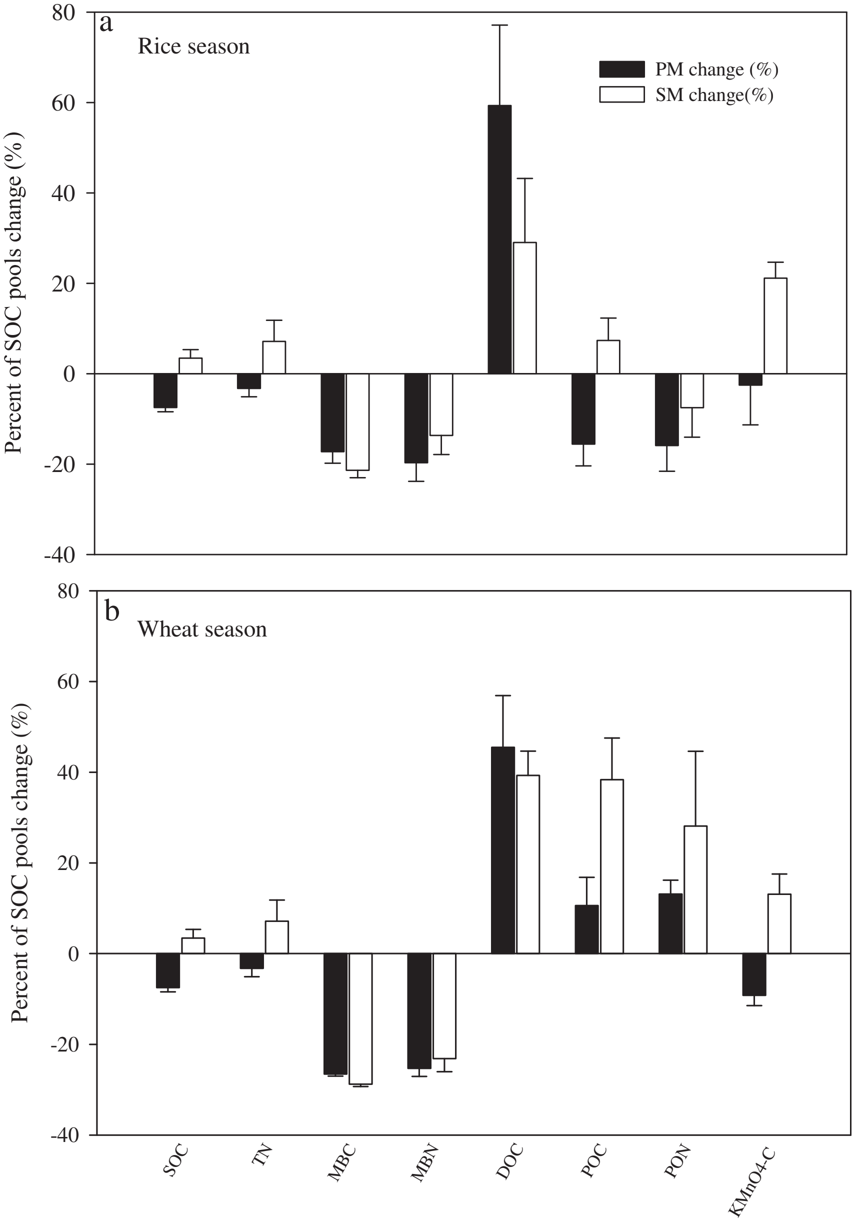

Fig. 3. Changes in soil organic C fractions under PM and SM versus TF after the rice season (a) and wheat season (b) at 0–5 cm soil depth. The changes were presented: (soil organic C fractions under PM or SM − soil organic C fractions under TF) × 100/soil organic C fractions under TF. Error bars represent standard errors of the means ($n = 3$). Abbreviations for treatments: TF = traditional flooding, PM = plastic film mulching, SM = wheat straw mulching.

on average almost 22% for the non-flooded mulching cultivation systems. In particular, the relative increase of the DOC fractions was almost 60% after the rice season, 46% after the wheat season.

The relative changes of labile SOM fractions within two cropping seasons differed in response to cultivation systems (Fig. 4). Compared with TF, POC and PON were the most sensitive indicators under PM and SM. POC accounted for 44.6% and 43.0% changes between two cropping seasons under PM and SM, while PON accounted for 63.8% and 69.2% under PM and SM.

4. Discussion

Compared with TF, non-flooded mulching leads to a similar (PM) or increase (SM) SOM concentration in the 0–5 cm topsoil in rice–wheat rotation (Table 2), confirming our previous findings [7,8]. The results showed that non-flooded mulching cultivation and cropping season had a great influence on labile SOM fractions. Since the largest differences were recorded in the upper 5 cm, with rare significant differences between 5–12 cm and 12–24 cm soil depths, the discussion focuses mainly on 0–5 cm depth.

Microbial biomass C only comprises about 1–5% of soil organic C, and microbial biomass N takes about 2–6% of total N, but they thought to exert a key controlling influence on the rate of nutrient cycling in agricultural ecosystems [20,21]. The significantly higher MBC and MBN concentrations in TF versus non-flooded mulching (PM and SM) in 0–5 cm were observed (Fig. 1). This supports the previous observation that PM reduced microbial biomass C and N but increased microbial biomass P compared with TF in three of five field experiments [22]. The lower microbial biomass in the non-flooded mulching system than traditional flooding system may indicate the difference in microbial community composition between aerobic and anaerobic condition. According to Kimura et al.

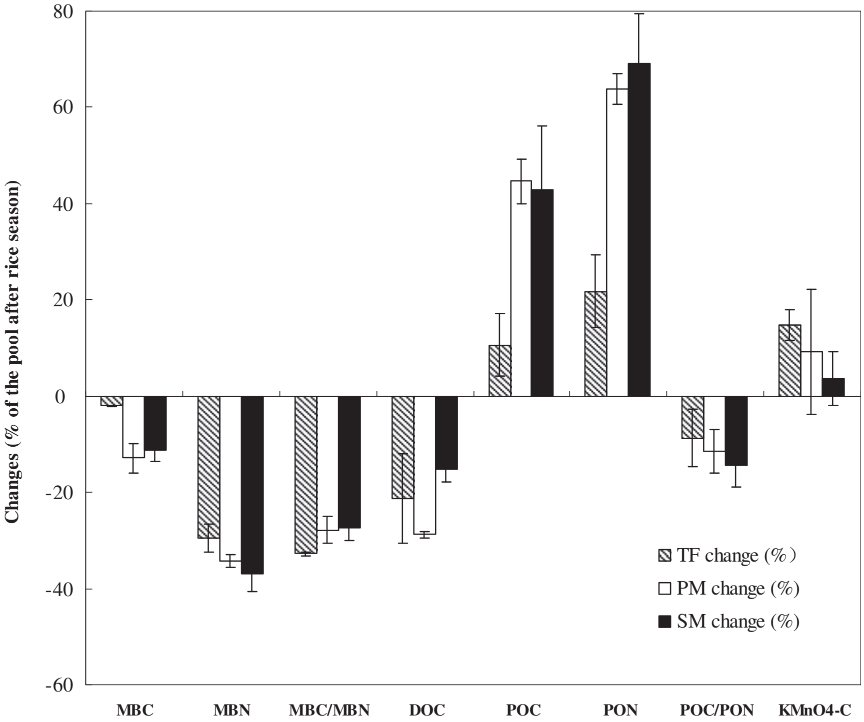

Fig. 4. Changes of soil organic C fractions following rice to wheat season under three cultivation systems at 0–5 cm soil depth. The changes are presented: (soil organic C fractions under three systems after wheat season − soil organic C fractions under three systems after rice season) × 100/soil organic C fractions under three systems. Error bars represent standard errors of the means ($n = 3$). Abbreviations for treatments: TF = traditional flooding, PM = plastic film mulching, SM = wheat straw mulching.

[23], microbial community structure of a flooded rice soil, inferred from PLFA analysis, differed from that in drained soil. Bossio and Scow [24] also observed a decrease in fungal and aerobic indicators and an increase in Gram-positive bacterial indicators under flooding as compared with non-flooded condition.

In contrast to MBC and MBN, TF showed a lower DOC concentration compared with non-flooded mulching. This probably reflects a lower microbial use and microbial production. DOC represents the main source of substrate for microorganisms; moreover, microbial metabolites also constitute a significant proportion of DOC [25,26]. Bacteria are commonly dominant under flooded conditions while fungi under upland conditions [27,28]. TF treatment may have a lower microbial production as fungi acts as important agents in the DOC production process, this probably because of incomplete degradation of organic matter by fungi [26]. $KMnO_4$-C reflects relatively younger and less recalcitrant organic compounds including labile humic materials and polysaccharides. POC is a transitory pool of organic matter between fresh residue and humified organic matter [9]. POC and $KMnO_4$-C concentrations increased under SM. This reflects a greater organic matter input (residue input) in this system.

Microbial biomass and DOC concentrations were higher for all three cultivations after the rice season than after the wheat season. This increase was caused by the seasonal temperature change, and higher temperature is known to stimulate biological activity, the decomposition of organic C and the release of DOC from soil organic matter [26,29]. Based on a one-year field experiment, Bonnett et al. [30] suggested that seasonal temperature significantly increased the DOC concentration. In contrast, POC under PM and SM was higher after the wheat season than after the rice season. POC reflects young organic compounds and is composed primarily of residue, litter, shallow roots and microbial and micro-faunal debris [9,31]. The increase of POC under PM and SM after the wheat season (compared with the rice season) could be a result of higher C input from crop, roots and rhizodeposition in the wheat season [32]. We did not measure root biomass under field conditions, so we estimated root biomass by using the ratio 0.19 (roots/aboveground biomass) for rice and 0.30 for wheat [33,34]. We found higher root biomass production after the wheat vs. rice season under both PM and SM systems (2970 vs. 2166 kg ha^{-1} for PM; 2961 vs. 1643 kg ha^{-1} for SM). Therefore, the seasonality of organic matter input to the soil was potentially the main factor affecting the amount of POC fractions. This agrees with Russell et al. [35], who also reported that plant species significantly differed in their effects on POC concentration.

Labile SOM fractions are associated with nutrient mineralization and can make an important contribution to nutrient availability, nutrient cycling and crop production. Nayak et al. [36] found a positive relationship between the sustainability yield index and POC concentration. In the same field study, Fan et al. [8] found that the average rice yields over 10 years were 6.2, 7.1 and 5.5 Mg ha^{-1} under TF, PM and SM, respectively. Thus, it seems that higher labile SOM fractions (DOC, POC, $KMnO_4$-C) under SM in the present study did not lead to positive effects on grain yield. However, increase in labile SOM fractions may imply environmental risk in C and N loss. For example, non-flooded mulching system decreased CH_4 emission but increased N_2O, and the global warming potential effects were even 9–10 times higher than traditional flooding system [37]. The increased labile fractions which provided easily decomposed C for microorganisms, such as higher DOC concentration under non-flooded mulching system while higher POC, $KMnO_4$-C contents under SM, should be responsible. In a laboratory experiment, Tian et al. [38] also found higher root-derived respiration (sum of roots and rhizomicrobial CO_2) under non-flooded rice systems as compared with traditional flooding condition. Nevertheless, a more mechanistic understanding of effects of specific labile organic matter fraction on greenhouse gas emission under non-flooded mulching systems still is required.

5. Conclusions

Labile SOM fractions were significantly affected in the top soil layer (0–5 cm) by long-term non-flooded mulching cultivation. Compared with traditional flooding system, a decreased trend of MBC and MBN, but increased DOC (PM and SM), POC and $KMnO_4$-C (SM) concentrations were observed under non-flooded mulching systems. Additionally, short-term effects of cropping seasons on labile SOM fractions were also evident. The DOC fraction was the most sensitive fraction affected by non-flooded mulching, while POC and PON fractions respond fast within the two cropping seasons. The further studies are still necessary for evaluation the effects of changes of specific SOM fractions under non-flooded mulching systems on soil productivity and environmental sustainability.

Acknowledgments

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